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RELATIVE CONTRIBUTION OF BREEDING SYSTEM AND ENDEMISM TO GENOTYPIC DIVERSITY: THE OUTCROSSING ENDEMIC *TARAXACUM CALIFORNICUM* VS. THE WIDESPREAD APOMICT *T. OFFICINALE* (*SENSU LATO*)

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ABSTRACT

We used allozymes to compare the population genetic structure of *Taraxacum californicum* Munz & I. M. Johnston, an outcrossing endemic of the San Bernardino Mountains of California, to that of its widespread weedy relative, *T. officinale* Wigg. (*sensu lato*), an obligate apomict. The average number of allozyme phenotypes per population of the endemic was six times that of the widespread species. The endemic had about twice as much genotypic diversity (estimated by *D*) per population than the widespread species, and that diversity was distributed much more evenly per population than in the widespread species. Both species had about the same level of average interpopulation differentiation. For this pair of related species, breeding system apparently plays a more important role than endemism in determining population genetic structure.

Restricted geographical distribution and small population size are characteristics of endemic plant species. Population genetic theory predicts that these conditions should influence population genetic structure, and, as a consequence, endemic species are expected to lack or to have reduced genetic polymorphism (reviewed by Ellstrand and Elam 1993). Empirical evidence that supports those theoretical expectations is accumulating; and genetic diversity tends to increase with species range size (e.g., Hamrick and Godt 1990; Karron 1991).

Breeding systems are also known to influence population genetic structure. Theory predicts that predominantly selfing and asexual species should exhibit lower variation within populations and greater interpopulation differentiation than outcrossers (Jain 1976; Baker 1959; Levin and Kerster 1971). Again, empirical data support these expected trends (e.g., Ellstrand and Roose 1987; Hamrick and Godt 1990).

It is not clear which factor, breeding system or endemism, should have a greater influence on population genetic structure. The answer to this question will be an important one for plant conservation managers who are rarely able to assess the genetic diversity of every species put in their charge. Such information will help managers judge under what circumstances loss of genetic variation might be most severe. For example, all other things being equal, if breeding system is more important than endemism, then outcrossing species will have more genetic variation to lose as their populations become increasingly fragmented under disturbance whereas clonal or selfing populations will tend to start with relatively low variation.

Population genetic comparisons of predominantly outcrossing endemic species and related selfing or asexual widespread species would reveal valuable information about the relative contributions of breeding system and endemism to population structure. If endemism is the more important factor, then genetic analyses should reveal a paucity of genetic polymorphism within populations of the endemic species compared to a widespread selfing or asexual congener. Alternatively, if breeding system influences population structure more than endemism, then within-population genotypic diversity of an outcrossing species with a restricted distribution should be greater than that of a selfing or asexual widespread species. And because interpopulation gene flow is typically higher in outcrossing species than a selfing or asexual species (Hamrick and Godt 1990), then we would expect that an outcrossing endemic should exhibit less interpopulation differentiation than a widespread selfing or asexual species.

A genus well suited for comparative study of the effects of endemism and breeding system on population genetic structure is *Taraxacum*. The great majority of species in this large genus are asexual, producing seeds that are genetically identical to the maternal parent through apomixis (agamospermy); and most of the remainder are predominantly or obligately outcrossing species (Grant 1981). The species we chose for study are the *T. californicum* Munz & I. M. Johnston (California dandelion) and

T. officinale Wigg. (common dandelion). Although *T. californicum* is in the section *Ceratophora* and *T. officinale* is in *Ruderalia*, the two species are so closely related that they occasionally spontaneously hybridize when they come in contact (Skinner and Pavlik 1994).

Taraxacum californicum is a predominantly outcrossing perennial herb endemic to moist, subalpine (1950–2400 m) meadows of the eastern San Bernardino Mountains of southern California (Munz 1974; Krantz 1980; Hickman 1993). The species was recently listed as endangered by the U.S. Fish and Wildlife Service (Federal Register 1998).

Taraxacum officinale, also a perennial herb native to Europe, is a pantemperate weed of lawns, meadows, and disturbed places (in California, from 0–3300 m); and, it occurs throughout North America (Munz 1974; Hickman 1993). The taxon is obligately apomictic. While treated as a single species by most North American researchers, *T. officinale* probably represents what many European experts judge to be a complex of agamospecies (Grant 1981; Richards personal communication). Like other North American researchers (Taylor 1987; King 1993), we were unable to identify any character that allowed for the easy assignment of separate taxa. Therefore, we consider the species to be "*sensu lato*".

The purpose of this study was to survey the genotypic diverity within and among populations of *T. califiornicum*, an outcrossing endemic, and *T. officinale*, its widespread apomictic congener. We compared their patterns of genetic diversity to distinguish the relative contributions of breeding system and endemism on their population genetic structure. Additionally, we add some baseline information on the genetic biology of *T. californicum* with bagging experiments and chromosome counts.

Methods and Materials

Collection of material. We used unopened flower buds for our genetic analysis. In the case of *T. californicum*, in June 1982 we collected one unopened flower bud from each of 30 randomly selected plants in each of 5 representative populations from its range in the eastern San Bernardino Mountains of California (Fig. 1). The buds were stored in plastic bags and kept cool until they could be extracted for allozyme study in Riverside. We also collected achenes to be germinated for chromosomal analysis from 3 individuals located in the population near Bluff Lake.

In the case of *T. officinale*, flower buds were obtained from plants grown from seed. During June and July 1980 we collected a mature infructescence (head) from each of 30 randomly selected plants in each of 22 *T. officinale* populations across the United States, detailed in Lyman and Ellstrand (1984). Heads were considered to be mature when they were fully opened, with achenes exposed to the wind. The heads were transported to Riverside for germination as described below.

In collecting both species, plants sampled were separated spatially ($\gg$1 m) to insure that rosettes were not attached to the same taproot (Naylor 1941). All collection sites were isolated from each other by at least 4 kilometers.

Germination. Achenes were germinated in 4-inch styrofoam cups in a greenhouse at the University of California at Riverside and later transferred to clay pots in the lathhouse. Germination occurred readily within 3–7 days. One seedling per maternal parent was grown to maturity for electrophoretic analysis. Additional seedlings were used for chromosome counts.

Chromosome counts. Chromosome numbers were counted for the offspring of five individuals from the Bluff Lake population of *T. californicum* and from two individuals from each population of *T. officinale*. We germinated seeds from these populations on moist filter paper in petri dishes under lab conditions. Four- to six-day-old root tips were placed in vials with 0.2% colchicine solution for 2 h and then fixed in 1:3 aceto-alcohol (Richards 1972a). The root tips were hydrolyzed in 0.1 N HCl for 11 minutes at 60°C and then stained in Feulgen solution for at least 1 h before the squashes were prepared (Löve and Löve 1975). We squashed 2-mm lengths of root tips on microscope slides in 1% aceto-propionic acid before viewing root tip meiotic cells under the microscope. A minimum of three metaphase plates per plant were examined and counted.

Testing for apomixis and autogamy in California dandelion. We bagged flowers of *T. californicum* to test whether it can set seed without the assistance of animal vectors (i.e., by apomixis or autogamy). In June 1982 at Wildhorse Meadows, the least disturbed site, small-mesh net bags were placed over one unopened capitulum on each of five plants of *T. californicum*. The bags were secured tightly to the ground with metal spikes to prevent insect movement in and out of the bags. In July the bags were removed, and each head was placed in a separate plastic bag so that seed set, as judged by whether achenes were fully developed and filled, could be determined in the laboratory.

Electrophoretic analysis. Unopened flower buds from up to 30 plants per population of *T. californicum* collected from the field were subjected to isozyme analysis by starch gel electrophoresis. For *T. officinale*, we analyzed unopened buds from up to 20 plants per population of plants grown from field-collected seed. Individuals buds were homogenized in two drops of extraction buffer (0.01 M DTT buffered with 0.1 M Tris-HCl, pH 7.0). Homogenates were adsorbed to paper wicks which were inserted vertically into 12% electrostarch gels. We used the Tris-EDTA-borate continuous gel and

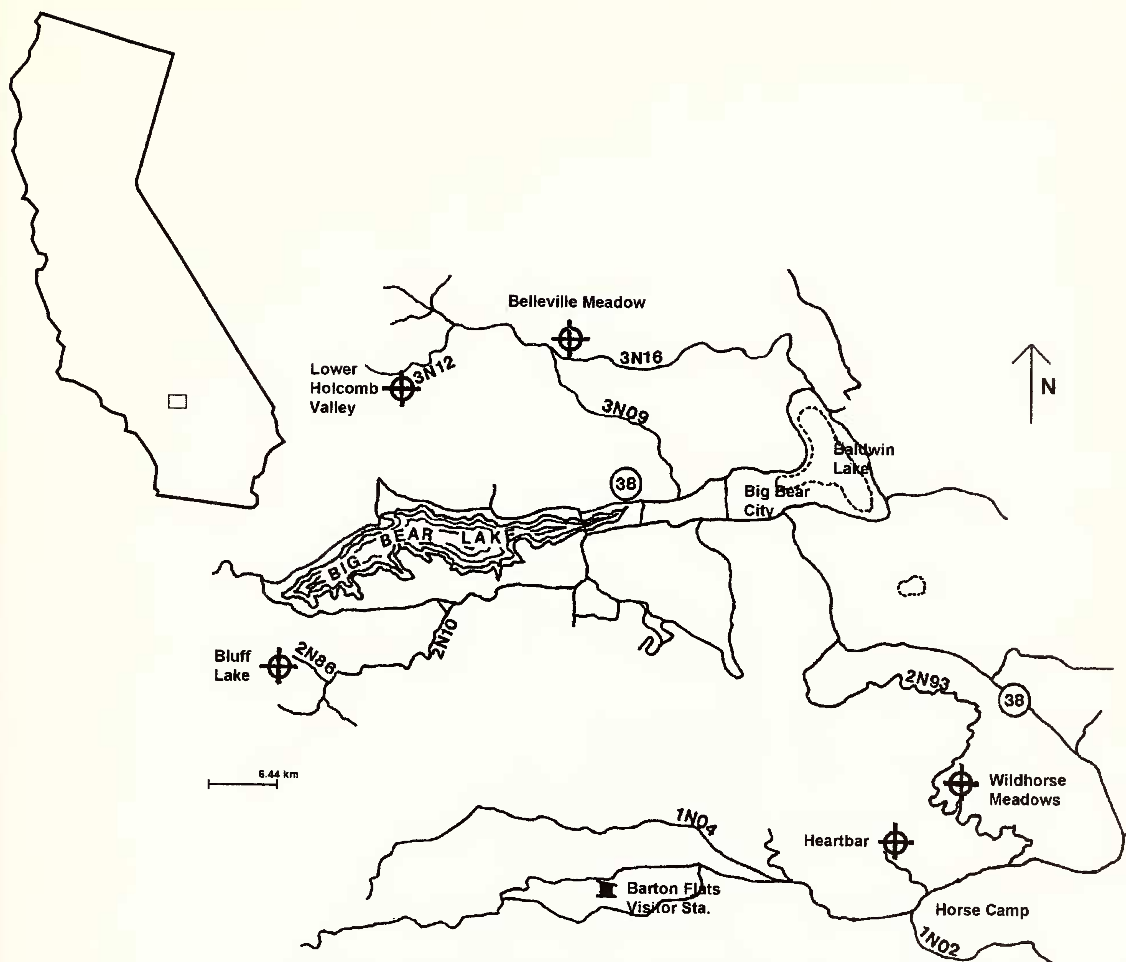

FIG. 1. Collection sites of 5 populations of *Taraxacum californicum*, San Bernardino Mountains, CA (inset area enlarged).

electrode buffer system of Heywood (1980). Electrophoresis was conducted for 4 h at 50 milliamps. Plastic containers of ice were placed on the gels during the run to prevent overheating. Internal standards were run on each gel to determine the electrophoretic equivalence of bands from different populations. We assayed for three enzymes—alcohol dehydrogenase (ADH), phosphoglucoisomerase (PGI), and phosphoglucomutase (PGM)—using the staining procedures described by Heywood (1980). Genetic analysis of isozyme patterns in related species (e.g., Roose and Gottlieb 1976) suggest that electrophoresis resolves five loci (one for ADH, two for PGI and PGM). Despite the polyploidy of these species (see Results), banding patterns were simple, and alleles were easily assigned.

RESULTS

Chromosome studies revealed a tetraploid chromosome complement in the nucleus of all individuals of *T. californicum* surveyed. In all counts, the number of chromosomes was 31 ($2n = 31$). Similar levels of unusual aneuploid tetraploidy have been reported in some sexual European species of *Taraxacum* (Richards 1972a, b, 1973). *Taraxacum officinale*, however, has been shown to be a triploid, $X = 8$, $2n = 24$ (Munz 1974). Our analysis of two individuals from each of 22 populations of this species showed the same chromosome number and ploidy level. Chromosome size was distinctly different in the two species. *Taraxacum californicum* chromosomes were observed to be more than twice the size of those of *T. officinale*.

Three of five bagged capitula produced no seed at all. The number of unfertilized ovules per head was 53, 73, and 76. Two remaining capitula produced a few filled achenes, 8 of 78 in one, and 2 of 67 in another. Overall, 2.9% of the ovules produced seed when foreign pollen was excluded. Unbagged inflorescences on the same plants had full seed set (100%). These data support the hypothesis that *T. californicum* is a self-incompatible outcrossing species.

TABLE 1. ALLELES IN *T. CALIFORNICUM* AND *T. OFFICINALE.*

Locus	Allele	Taxon	
		T. californicum	*T. officinale*
PGI-1	a	x	x
	b	x	x
	c	x	x
	d	x	x
	e	x	
	f	x	
PGM-1	a	x	x
	b		x
	c	x	x
	d	x	x
	e		x
	f	x	
ADH	a	x	x
	b	x	x
	c	x	x
	d		x
	e	x	

Genotypic variation was present within and among the populations of *T. californicum* and *T. officinale* investigated. We report genotypic rather than allele frequency data throughout this study because dosage effects due to polyploidy in both species were not clearly discernible on the starch gels, making it impossible to determine allele frequencies. Levels of variation were different for the two species. Of the five loci considered, two were monomorphic for both *T. officinale* and *T. californicum*. The polymorphic loci were shared by both species. Some alleles were common to both species, whereas others were species-specific (Table 1). We found 56 unique genotypes (allozyme phenotypes) among 147 individuals of *T. californicum* surveyed. The range of genotypes per population was 17–19 ($\bar{x}$ = 18.2; Fig. 2). No population contained more than six individuals of the same genotype. In contrast, only 21 genotypes were found among the 518 individuals surveyed, ranging from 1–9 ($\bar{x}$ = 3.2) genotypes per population (Fig. 2).

Pielou's correction version of the Gini Index (*D*) describes the degree of diversity within a population (cf. Ellstrand and Roose 1987). This index emphasizes changes in common rather than rare classes. *D* can be as little as 0 (a uniform sample) or as large as 1 (every individual different). The corrected values for the five populations of *T. californicum* ranged from 0.94–0.97 ($\bar{x}$ = 0.96). Values for *T. officinale* ranged from 0.00–0.89 ($\bar{x}$ = 0.50). Evenness values (*E*), which reflect how evenly the genotypes within a population are distributed among individuals, were also calculated (Fig. 3) (cf. Ellstrand and Roose 1987). This value also ranges from zero (extreme skewness) to 1.0 (complete equitability). The striking bimodal distribution of *E* for *T. officinale* showed that in some populations one or a few dominant clones predominated but that in the

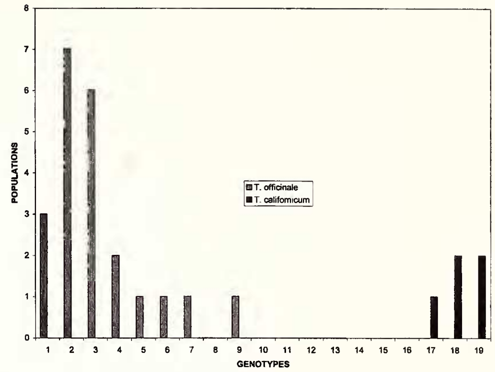

FIG. 2. Number of allozyme phenotypes per population for *T. californicum* and *T. officinale*.

others, the clones were equitably distributed among the individuals of the population. All five *T. californicum* populations, however, had evenness values close to 1.0 (Fig. 3). Both evenness and diversity differed significantly between these species ($P < 0.01$; Mann-Whitney U test).

Interpopulational differentiation within each species was measured using Hedrick's (1971) formula for genotypic similarity. This index is as follows:

$$I_{xy} = \frac{\sum_{j=1}^{n} P_{jx}P_{jy}}{\frac{1}{2}\left(\sum_{j=1}^{n} P_{jx}^2 + \sum_{j=1}^{n} P_{jy}^2\right)}.$$

The values for this formula range from 0 (no similarity among populations) to 1 (complete genotypic identity among populations). The values of the five populations of *T. californicum* were uniformly high, from 0.70–0.96 ($\bar{x}$ = 0.83). The values for the 22 populations of *T. officinale* ranged from 0.35–1.0 ($\bar{x}$ = 0.81).

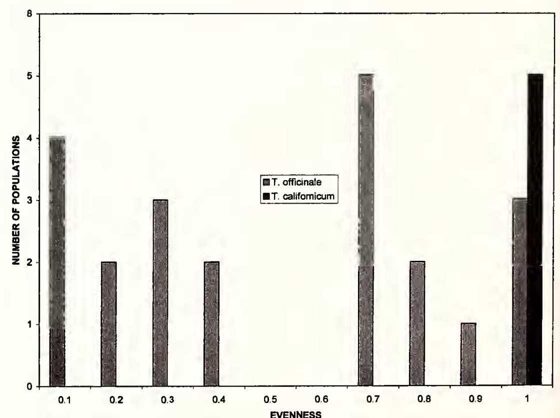

FIG. 3. Evenness values per populations for *T. officinale* and *T. californicum*.

Discussion

In our comparison of two congeners having different recombination systems and range sizes, we found that the predominantly outcrossing endemic species *T. californicum* had substantially more genotypic diversity than the apomictic and widespread species *T. officinale* (*sensu lato*). The average number of allozyme phenotypes per population of the endemic was six times that of the widespread species. Likewise, the endemic had about twice as much genotypic diversity per population as the widespread species, and that diversity was distributed much more evenly per population than in the widespread species.

Both species had about the same level of average interpopulation differentiation as estimated by Hedrick's *I*. The similarity is curious, given that the two species were sampled over such different scales (*T. californicum* over a scale of tens of kilometers; *T. officinale* over hundreds of kilometers). We would expect much more interpopulation differentiation in *T. officinale* for two reasons. First, we would expect more isolation (and consequently more differentiation) among distantly sampled populations compared to those sampled at a finer scale. Second, as noted above, all other things being equal, we would expect an apomictic species to have reduced gene flow relative to a sexual species (and consequently more differentiation). Gene flow by seed is the only means available to those species that reproduce without fertilization, whereas sexual species can disperse their genes by both seed and pollen. The best interpretation we can make is that the level of isolation among the meadows that make the home of the California dandelion are roughly the same as the level of isolation among our widely sampled common dandelion populations.

Results from our bagging experiment are compatible with an outcrossing breeding system for *T. californicum* based on self-incompatibility. Bagged capitula set few or no seed; unbagged capitula on the same plants had full seed set. Although this species may be pseudogamous or semigamous, requiring pollination to stimulate apomictic seed production (Richards 1986), such syndromes are unknown for *Taraxacum* (Grant 1981). Also, net bags might have raised the temperature of the capitula to the point that apomictic seed production was disrupted (Stebbins personal communication). However, as noted above, self-incompatible, endemic montane *Taraxacum* are known in Europe (Richards 1973), and apomixis requiring pollination has never been reported for the genus (Grant 1981). Finally, the high genotypic diversity we discovered in *T. californicum* argues against high levels of apomictic seed production. Prior genetic surveys of agamospermous species typically show much lower genotypic diversity (e.g., Ellstrand and Roose 1987; Diggle et al. 1998). We acknowledge that demonstrating sexuality conclusively requires further experimentation, but, presently, all of the evidence supports outcrossing as *T. californicum*'s breeding system.

We recognize that using a small number of marker loci can underestimate genotypic diversity. Adding more characters may identify more genotypes (Ellstrand and Roose 1987). Nonetheless, it is clear that the two species have different population genetic structures for the same set of genetically based markers. Genotypic diversity in *T. officinale* is low, but virtually every individual of *T. californicum* was found to be genotypically distinct. Indeed, we are confident that if we were able to add a few more markers, we would be able to distinguish among those few individuals of *T. californicum* that shared a genotype in this study.

For this pair of species, the difference in breeding system is apparently more important in determining population genetic structure than the difference in range size. The relatively high levels of genotypic diversity persist in the apparently outcrossing *T. californicum* despite its limited geographic range. We are aware of only one other study that compares an endemic, outcrossing species with a widespread species that has an alternative breeding system. The self-incompatible endemic of New Mexico's Organ Mountains, *Oenothera organensis* Munz, is nearly monomorphic at several allozyme loci despite high polymorphism at self-incompatibility locus (Levin et al. 1979). Most of its congeners that have been studied have more genetic diversity (Levin et al. 1979), and almost all of these have an essentially clonal reproductive system (permanent translocation heterozygosity; Grant 1981). Thus, the trend in *Oenothera* is opposite that found here for *Taraxacum*.

Taraxacum californicum is believed to be a relict of the section *Ceratophora* (Jepson 1925) that is thought to have spread through the northern hemisphere during an interglacial period (Richards 1973). Fossil records of the section date to 1×10^5 years B.P. (Chearney and Mason 1936). Subsequently *T. californicum* is presumed to have become isolated from the many other species of the section, which occur in a circumpolar distribution (Richard 1973). Given its long isolation, the fact that its total population size may be less than 6×10^3 plants, and its restricted habitat, the polymorphism of *T. californicum* is surprisingly high.

The remarkable genetic diversity that *T. californicum* exhibits despite its endemism suggests that breeding system plays a greater role in maintenance of genetic variation than the constraints of endemism do to limit it. But there is another possibility to account for the origin and maintenance of genetic variation. The ploidy difference between *T. californicum* and *T. officinale* may explain at least some of the greater genotypic variation displayed in *T. californicum*. If this species does, in fact, belong to the section *Ceratophora*, then, like the tetraploid European members of this section, it is

probably an allotetraploid derived from two diploid sexual species (Richard 1973). The resulting sexual amphiploid will initially breed true for a highly heterozygous genotype but will release that variation slowly over generations through rare tetrasomic recombination (Grant 1981; Roose and Gottlieb 1976).

Another possibility is that *T. californicum* is not yet in evolutionary equilibrium. If fragmentation and endemism are relatively recent in terms of the mean generation time of the species, the patterns we observed better represent historic inertia than the factors that are currently molding patterns of diversity (Ellstrand and Elam 1993). Because the species was only described in this century, we know little of its history. If *T. californicum* population genetic structure is not in equilibrium, we might expect to see the effects of isolation and endemism eventually work to erode the current levels of genetic diversity, but such changes could take decades.

A puzzling feature of the chromosome studies is the aneuploid condition found in the individuals of *T. californicum* at Bluff Lake. Investigators have noted the same phenomenon in a number of European *Taraxacum* species (Malecka 1962, 1967a, b, 1969; Sorensen and Gudjonsson 1946; Richards 1970, 1972a, b, 1973), including many sexual species (e.g., Sorensen and Gudjonsson 1946). The extent of the aneuploid condition and its role in reproductive events in *T. californicum* are presently unknown.

This research has uncovered relatively high levels of genotypic variation in an endemic species in comparison with its widespread apomictic congener. We conclude that endemics need not necessarily be genotypically depauperate species relative to widespread congeners. Instead, it is clear that the organization of genetic variation may be subject to other constraints such as breeding system and history. Further descriptive and experimental population genetics studies are needed for this and other species pairs to determine the nature of these constraints.

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